ACTIVE HAIR-BUNDLE MOTILITY HARNESSES NOISE TO OPERATE NEAR AN OPTIMUM OF MECHANOSENSITIVITY

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The ear relies on nonlinear amplification to enhance its sensitivity and frequency selectivity. In the bullfrog's sacculus, a hair cell can mobilize active oscillatory movements of its hair bundle to amplify its response to faint stimuli. Hair-bundle oscillations can result from an interplay between a region of negative stiffness in the bundle's force-displacement relation and the Ca^{2+}-regulated activity of molecular motors. Within the framework of this simple model, we calculate a state diagram which describes the possible dynamical states of the hair bundle in the absence of fluctuations. Taking different sources of fluctuations into account, we find conditions that yield response functions and spontaneous noisy movements of the hair bundle in quantitative agreement with experiments. We show that fluctuations restrict the bundle's sensitivity and frequency selectivity but find that a hair bundle studied experimentally operates near an optimum of mechanosensitivity in our state diagram.

1 Introduction

The mechanosensory hair cells of the vertebrate ear amplify their inputs to enhance sensitivity and frequency selectivity to weak oscillatory stimuli (reviewed in [1]). Although the cellular mechanisms that mediate this active process have remained elusive, *in vitro* [2] as well as *in vivo* [3] experiments have revealed that the mechanosensory organelle of the hair cell - the hair bundle - can generate active oscillatory movements. When mechanically stimulated near its frequency of spontaneous oscillation, a hair bundle displays a compressive nonlinearity that demonstrates amplified responses to faint stimuli [4]. It has been noticed that this behavior resembles that of dynamical systems that operate in the vicinity

of an oscillatory instability, a Hopf bifurcation (ref. [4] and references therein).

Hair-bundle oscillations are noisy [5]. Noise blurs the distinction between active oscillations and fluctuations and thus conceals the bifurcation between oscillatory and non-oscillatory states. We present here a theoretical description of the effects of fluctuations on active hair-bundle motility.

2 Models and Results

2.1 Active Hair-Bundle Mechanics

Active hair-bundle oscillations are most convincingly explained by an interplay between a region of negative stiffness in the bundle's force-displacement relation and the Ca^{2+}-regulated activity of the molecular motors that mediate mechanical adaptation [6]. This interplay can be described by two coupled equations:

$$\lambda \dot{X} = -K_{\text{gs}}(X - X_a - DP_o) - K_{\text{sp}}X + F_{\text{ext}} + \eta \quad , \qquad (1)$$

$$\lambda_a \dot{X}_a = K_{\text{gs}}(X - X_a - DP_o) - \gamma f_{\text{max}}(1 - SP_o) + \eta_a \quad . \qquad (2)$$

Eq. **1** describes the dynamics of the hair-bundle position X. The hair bundle is subjected to friction, characterized by the coefficient λ, as well as to the elastic forces $-K_{sp}X$ and $-K_{gs}Y$, where K_{sp} and K_{gs} are the stiffness of stereociliary pivots and that of the gating springs, respectively, and to the external force F_{ext}. The open probability of transduction channels is P_o. Channel opening reduces the gating-spring extension by a distance D. Active hair-bundle movements result from forces exerted by a collection of molecular motors within the hair bundle. By adjusting the gating-spring extension, these motors mediate mechanical adaptation to sustained stimuli (reviewed in [7]). The variable X_a can be interpreted as the position of the motor collection. Eq. **2** describes the mechanics and the dynamics of these motors by a linear force-velocity relation of the form $\lambda_a dX_a/dt = -F_0 + F_{\text{mot}}$, where λ_a characterizes the slope of the force-velocity relation. In the hair bundle, the motors experience an elastic force $F_{\text{mot}} = K_{gs}Y$. At stall, these motors produce an average force $F_0 = \gamma f_{\text{max}}(1 - SP_o)$, where $\gamma \simeq 1/7$ is a geometric projection factor, f_{max} is the maximum force that the motors can produce and S represents the strength of Ca^{2+} feedback on the motor activity [8]. This last parameter is expected to be proportional to the Ca^{2+} concentration in endolymph [6]. Here we assumed that calcium dynamics at the motor site is much faster than hair-bundle oscillations. Active force production by the motors corresponds to motors climbing up the stereocilia, i.e. $dX_a/dt < 0$. In a two-state model for channel gating, the open probability can be written as

$$P_o = \frac{1}{1 + Ae^{-(X-X_a)/\delta}} \quad , \qquad (3)$$

where $A = \exp([\Delta G + (K_{gs}D^2)/(2N)]/k_B T)$ accounts for the intrinsic energy difference ΔG between the open and the closed states of a transduction channel and $\delta = N k_B T/(K_{gs}D)$.

2.2 State Diagram in the Absence of Noise

To explore the dynamic behaviors of the system described by Eqns. **1-2**, we first ignore the effects of fluctuations and assume $F_{\text{ext}} = 0$. Steady states satisfy $dX/dt = 0$ and $dX_a/dt = 0$. Linear stability analysis of these steady states reveals conditions for stability as well as for oscillatory instabilities that lead to spontaneous oscillations via a Hopf bifurcation. The state diagram exhibits different regimes (Fig. 1). If the force f_{max} is small, the motors are not strong enough to pull transduction channels open. In this case, the system is monostable with most of the channels closed. Increasing f_{max} leads to channel opening. For intermediate forces and weak Ca^{2+} feedbacks, the system is bistable, i.e. open and closed channels coexist. For strong Ca^{2+} feedbacks, however, the motors can't sustain the forces required to maintain the channels open. Spontaneous oscillations occur in a region of both intermediate forces and feedback strengths. Note that there is no oscillation in the absence of Ca^{2+} feedback, i.e. for $S = 0$.
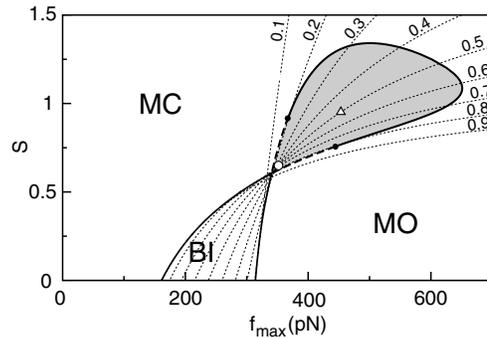

Figure 1. State diagram of a hair bundle. Lines of equal open probability of the transduction channels (dotted lines) are superimposed and are each indexed by the corresponding value. The hair bundle can be monostable with transduction channels mostly closed (MC) or mostly open (MO), bistable (BI) or oscillatory (grey area).

2.3 Effects of Fluctuations

Noise terms η, η_a in Eqns. **1-2** formally take into account the effects of various sources of fluctuations on X and X_a, respectively. Noise terms are zero on average. Their strengths are characterized by autocorrelation functions, respectively $< \eta(t)\eta(0) >$ and $< \eta_a(t)\eta_a(0) >$. We assume that different noise sources are uncorrelated and that noise is Gaussian.

Assuming that the motors are deactivated ($f = 0$), we first discuss thermal contributions to the noise. The noise term η in Eq. **1** then results from brownian motion of fluid molecules which collide with the hair bundle and from thermal transitions between open and closed states of the transduction channels. By changing the gating-spring extension, this channel clatter generates fluctuating forces on the stereocilia. The fluctuation-dissipation theorem implies that $< \eta(t)\eta(0) >= 2k_BT\lambda\delta(t)$. The friction coefficient $\lambda = \lambda_h + \lambda_c$ results from two contributions: $\lambda_h \simeq 1.3 \ 10^{-7} \mathrm{N \cdot s \cdot m^{-1}}$ accounts for hydrodynamic friction, which depends on bundle geometry and fluid viscosity [9], whereas λ_c results from channel clatter. The contribution λ_c can be estimated from the autocorrelation function of the force η_c that results from stochastic opening and closing of N transduction channels

$$< \eta_c(t)\eta_c(0) > \simeq D^2 K_{\mathrm{gs}}^2 P_o(1 - P_o)N^{-1}e^{-|t|/\tau_c} \simeq 2D^2 K_{\mathrm{gs}}^2 P_o(1 - P_o)N^{-1}\tau_c\delta(t) \quad . \tag{4}$$

Assuming that $< \eta_c(t)\eta_c(0) > \simeq 2k_BT\lambda_c\delta(t)$, we define a hair bundle friction λ_c which is associated to channel opening and closing. Using Eq. **4**, we estimate

$$\lambda_c \simeq \frac{K_{\mathrm{gs}}^2 D^2 P_o(1 - P_o)\tau_c}{Nk_BT} \quad . \tag{5}$$

Using typical parameter values (see Table 1 in ref. [10]) our estimate reveals that channel clatter dominates friction and $\lambda \simeq 3 \ 10^{-6} \mathrm{N \cdot s \cdot m^{-1}}$.

The noise strength resulting from stochastic motor action can also be estimated. Measurements of the initial adaptation rate as a function of the magnitude of step stimuli [8] imply that $\lambda_a \simeq 1.3 \ 10^{-5} \mathrm{N \cdot s \cdot m^{-1}}$. The stochastic activity of motors generates an active contribution η_m to η_a with

$$< \eta_m(t)\eta_m(0) > \simeq \gamma^2 N_a p(1 - p)f^2 e^{-|t|/\tau_a} \simeq 2N_a\gamma^2 p(1 - p)f^2\tau_a\delta(t) \quad . \tag{6}$$

Each motor can produce a force f and has a probability p to be bound. Here we have assumed that the N_a motors fluctuate independently and that relevant time scales for a hair-bundle oscillation are longer than τ_a which is the characteristic time of force production by the motors. This noise strength can be described by introducing an effective temperature T_m defined by $< \eta_m(t)\eta_m(0) > \simeq 2k_BT_m\lambda_a\delta(t)$. With $f \simeq 1\mathrm{pN}$, $\tau_a \simeq 10\mathrm{ms}$ and $p \simeq 0.05$, we find $T_m/T \simeq N_a\gamma^2 p(1 - p)f^2\tau_a/(k_BT\lambda_a) \simeq 0.5$. Writing $< \eta_a(t)\eta_a(0) >= 2k_BT_a\lambda_a\delta(t)$, we thus get $T_a \simeq 1.5T$.

2.4 Linear and Nonlinear Response Functions

Stochastic simulations of Eqns. **1-2** allow us to calculate linear and nonlinear response functions of the model in the presence of periodic force stimuli [10]. The

only free parameters are the Ca^{2+}-feedback strength S and the maximal motor force $f_{\max}$. Along a line of constant open probability $P_o = 0.5$, the characteristic frequency of spontaneous oscillations varies between a few Hertz and about 50Hz in the range $f_{\max} = 330 - 800\text{pN}$ within which a peak was detected in the spectral density of spontaneous movements. We elected the value of the motor force $f_{\max} \simeq 352\text{pN}$ (see $\circ$ in Fig. 1) at which the linear response function had the same shape as that observed experimentally [5]. At this operating point, the system displayed noisy spontaneous oscillations $X(t)$ that are similar to the hair-bundle oscillations observed in the bullfrog's sacculus [5,10].

The calculated linear response function χ_0 as a function of frequency agrees quantitatively with the experimental observations [5]. At the characteristic frequency of the spontaneous oscillations, the sensitivity of the system to mechanical stimulation exhibits the three regimes observed experimentally [4] as a function of the stimulus amplitude (Fig. $2C$): a linear regime of maximal sensitivity $|\hat{\chi}_0| = 8.5\text{km·N}^{-1}$ at $\omega = \omega_0$ for small stimuli, a compressive nonlinearity for intermediate stimuli and a linear behavior of low sensitivity for large stimuli. The maximal sensitivity as well as the breadth of the nonlinear region are in quantitative agreement with experiments. An important parameter that influenced the system's maximal sensitivity is the stiffness of the load to which the hair bundle is coupled. For $f_{\max} \simeq 352\text{pN}$, power spectra of spontaneous oscillations and response functions were not significantly affected by varying P_o in the range 0.2-0.8. Agreement between simulations and experiments thus did not qualify a particular value of P_o.
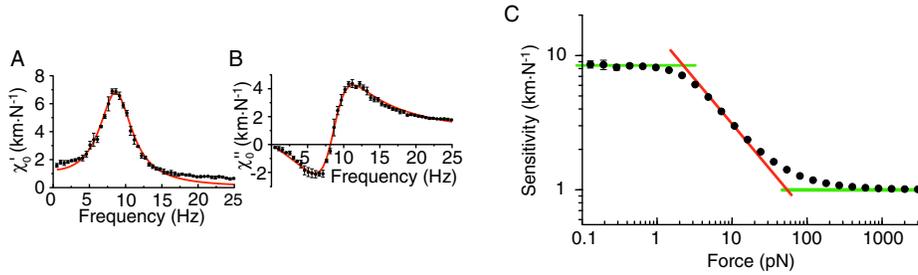

Figure 2. Response functions calculated from stochastic simulation of Eqns. **1-2** in presence of a periodic stimulus force. (A) Real part χ_0' of the linear response function. (B) Imaginary part χ_0'' of the linear response. (C) nonlinear response function at a fixed frequency (8Hz) near that of the system's spontaneous oscillations.

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3 Discussion

We have presented a physical description of active hair-bundle motility that emphasizes the role played by fluctuations. The mechanical properties of oscillatory hair bundles in the presence of a periodic stimulus force can be described quantitatively only if fluctuations are taken into account. In the absence of fluctuations, an operating point on the line of Hopf bifurcations in the state diagram would result in diverging sensitivity, infinite frequency selectivity and a compressive nonlinearity over many decades of stimulus magnitudes. This situation is ideal for detecting oscillatory stimuli [11,12,13]. As exemplified by our analysis, fluctuations restrict the system's sensitivity and frequency selectivity to oscillatory stimuli as well as the range of stimulus magnitudes over which the compressive nonlinearity of the bundle's response occurs. Despite fluctuations, a single hair bundle amplifies its response to small stimuli and, correspondingly, the characteristic compressive nonlinearity that arises near a Hopf bifurcation remains. One can define the gain of the amplificatory process as the ratio of the sensitivity at resonance to small stimuli and that to intense stimuli. Both experiments and simulations indicate that active hair-bundle motility provides a gain of about ten. Our theoretical analysis demonstrates that significant amplification happens inside the area of the state diagram where the noiseless system oscillates [10]. Interestingly, the global optimum of mechanosensitivity is obtained at an operating point located near the center of the oscillatory region in the state diagram (see $\triangle$ in Fig.1), thus far from the line of Hopf bifurcations of the noiseless system. Furthermore, the sensitivity is largest if the open probability of the transduction channels is 0.5.

The ability of a single hair bundle to detect oscillatory stimuli using critical oscillations is limited by fluctuations which conceal the critical point. This limitation could be overcome if an ensemble of hair cells with similar characteristic frequencies were mechanically coupled, as they probably are in an intact organ.

Acknowledgments

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Comments and Discussion

M. van der Heijden: The dynamical range of the mechanisms you describe is limited to 20 dB or so at the lower end. Of course, one could invoke "shifts in the operating point" to extend the dynamical range. But that seems to serve no practical purpose. Compression on the BM extends to very high sound levels, but you don't need ears to perceive such loud sounds. There exists no noise problem at 85 dB SPL - if anything those poor hair bundles should be protected against the destructive effects of the acoustic power. Do you really think that the reaction forces of these tiny transducer channels operate over a dynamic range of 80 dB, that is, a 100,000,000-fold power range?

Answer: In the absence of noise, a dynamical system that operates near an oscillatory instability becomes arbitrarily sensitive as the magnitude of the sinusoidal stimulus becomes smaller (provided that the system is stimulated at the characteristic frequency of the instability). If there were no noise, it would thus be no problem to get a dynamic range of 80 dB with the mechanism that produces active hair-bundle oscillations. Fluctuations restrict the range of the compressive nonlinearity by limiting the sensitivity to LOW stimuli but have no effect for intense stimuli. With fluctuations included, there is no operating point that yields the essential compressive nonlinearity, which characterizes the noiseless system. In fact, we suggest that the oscillatory hair bundles that we have studied operate near an optimum of mechanosensitivity; hence one would NOT observe a dramatic extension of the dynamical range by shifting the operating point. In an intact organ in vivo, the limiting effects of noise might be circumvented if the hair-bundle oscillator were coupled to other oscillators of similar characteristic frequencies, either within the same hair cell or in neighboring hair cells.

B. Brownell: What effect would a change in temperature have in your model and do your experiments show the predicted effect?

Answer: All our experiments have been done at room temperature and we have thus not tested the effect of temperature on a bundle's sensitivity to oscillatory stimuli.

R. Chadwick: Does a hair-bundle need to be in the oscillatory portion of the state diagram in order to exert an active force?

Answer: A hair bundle that operates in the stable regions of the state diagram (Fig 1) can indeed exert active forces in response to an external stimulus. Within the framework of our model, an external stimulus (a step force for instance) evokes an active movement of molecular motors that affects the tension in elastic gating springs, in turn producing a force on the hair bundle. Our analysis shows, however, that, in the presence of noise, the system is more sensitive to small oscillatory stimuli if it operates within the oscillatory region of the state diagram.